

**BIBLIOGRAPHY**

**General**

1. Dutton, G. AntiCancer Inc. scientists identify a key governing step in the metastasis of cancer. Genetic Engineering News 16, 1, January 15, 1996.
2. Holzman, D. Of mice and metastasis: A new for-profit model emerges. Journal of the National Cancer Institute 88, 396-397, 1996.
3. Riordan, T. A technique is said to ease attachment of tumors to mice, making them 'little cancer patients.' New York Times, "Patents" Column, March 4, 1996.
4. Dove, A. Illuminating cancer models. Nature Biotechnology 18, 261, 2000.
5. McCann, J. Jellyfish protein gives new glow to tumor imaging. Journal of the National Cancer Institute 93, 976-977, 2001.

**Bladder**

1. Fu, X., Theodorescu, D., Kerbel, R.S., and Hoffman, R.M. Extensive multi-organ metastasis following orthotopic onplantation of histologically-intact human bladder carcinoma tissue in nude mice. Int. J. Cancer 49, 938-939, 1991.
2. Fu, X., and Hoffman, R.M. Human RT-4 bladder carcinoma is highly metastatic in nude mice and comparable to *ras*-H-transformed RT-4 when orthotopically onplanted as histologically intact tissue. Int. J. Cancer 51, 989-991, 1992.
3. Chang, S-G., Kim, J.I., Jung, J-C., Rho, Y-S., Lee, K-T., An, Z., Wang, X., and Hoffman, R.M. Antimetastatic activity of the new platinum analog {Pt(cis-dach)(DPPE) $\cdot$ 2NO $_3$ } in a metastatic model of human bladder cancer. Anticancer Res. 17, 3239-3242, 1997.
4. Zhou, J-H., Rosser, C.J., Tanaka, M., Yang, M., Baranov, E., Hoffman, R.M., Benedict, W.F. Visualizing superficial human bladder cancer cell growth *in vivo* by green fluorescent protein expression. Cancer Gene Therapy 9, 681-686, 2002.

**Breast**

1. Fu, X., Le, P., and Hoffman, R.M. A metastatic orthotopic-transplant nude-mouse model of human patient breast cancer. Anticancer Res. 13, 901-904, 1993.
2. Kubota, T., Inoue, S., Furukawa, T., Ishibiki, K., Kitajima, M., Kawamura, E., and Hoffman, R.M. Similarity of serum - Tumor pharmacokinetics of antitumor agents in man and nude mice. Anticancer Res. 13, 1481-1484, 1993.
3. Li, X-M., Wang, J-W., An, Z., Yang, M., Baranov, E., Jiang, P., Sun, F-X., Moossa, A.R., and Hoffman, R.M. Optically imageable metastatic model of human breast cancer. Clinical & Experimental Metastasis, 19, 347-350, 2002.
4. John, C.M., Leffler, H., Kahl-Knutsson, B., Svensson, I., Jarvis, G.A. Truncated Galectin-3 Inhibits Tumor Growth and Metastasis in Orthotopic Nude Mouse Model of Human Breast Cancer. Clinical Cancer Research 9, 2374-2383, 2003.
5. Goodison, S., Kawai, K., Hihara, J., Jiang, P., Yang, M., Urquidi, V., Hoffman, R.M., and Tarin, D. Prolonged dormancy and site-specific growth potential of cancer cells spontaneously disseminated from non-metastatic breast tumors revealed by labeling with green fluorescent protein. Clinical Cancer Res. 9, 3808-3814, 2003.

**Colon**

1. ✓ Fu, X., Besterman, J.M., Monosov, A., and Hoffman, R.M. Models of human metastatic colon cancer in nude mice orthotopically constructed by using histologically intact patient specimens. Proc. Natl. Acad. Sci. USA 88, 9345-9349, 1991.
2. ✓ Fu, X., Herrera, H., Kubota, T., and Hoffman, R.M. Extensive liver metastasis from human colon cancer in nude and SCID mice after orthotopic onplantation of histologically-intact human colon carcinoma tissue. Anticancer Res. 12, 1395-1398, 1992.

3. Furukawa, T., Kubota, T., Watanabe, M., Kuo, T.H., Kase, S., Saikawa, Y., Tanino, H., Teramoto, T., Ishibiki, K., Kitajima, M., and Hoffman, R.M. Immunotherapy prevents human colon cancer metastasis after orthotopic onplantation of histologically-intact tumor tissue in nude mice. *Anticancer Res.* 13, 287-291, 1993.
4. Kuo, T-H., Kubota, T., Watanabe, M., Furukawa, T., Teramoto, T., Ishibiki, K., Kitajima, M., and Hoffman, R.M. Early resection of primary orthotopically-growing human colon tumor in nude mouse prevents liver metastasis: further evidence for patient-like hematogenous metastatic route. *Anticancer Res.* 13, 293-297, 1993.
5. Furukawa, T., Kubota, T., Watanabe, M., Kuo, T-H., Nishibori, H., Kase, S., Saikawa, Y., Tanino, H., Teramoto, T., Ishibiki, K., and Kitajima, M. A metastatic model of human colon cancer constructed using cecal implantation of cancer tissue in nude mice. *Jpn. J. Surg.* 23, 420-423, 1993.
6. Wang, X., Fu, X., Brown, P.D., Crimmin, M.J., and Hoffman, R.M. Matrix metalloproteinase inhibitor BB-94 (Batimastat) inhibits human colon tumor growth and spread in a patient-like orthotopic model in nude mice. *Cancer Res.* 54, 4726-4728, 1994.
7. Togo, S., Shimada, H., Kubota, T., Moossa, A.R., Hoffman, R.M. "Seed" to "soil" is a return trip in metastasis. *Anticancer Res.* 15, 791-794, 1995.
8. Togo, S., Shimada, H., Kubota, T., Moossa, A.R., Hoffman, R.M. Host organ specifically determines cancer progression. *Cancer Res.* 55, 681-684, 1995.
9. Kuo, T-H., Kubota, T., Watanabe, M., Furukawa, T., Teramoto, T., Ishibiki, K., Kitajimi, M., Moossa, A.R., Penman, S., Hoffman, R.M. Liver colonization competence governs colon cancer metastasis. *Proc. Natl. Acad. Sci. USA* 92, 12085-12089, 1995.
10. Togo, S., Wang, X., Shimada, H., Moossa, A.R., and Hoffman, R.M. Cancer seed and soil can be highly selective: Human-patient colon tumor lung metastasis grows in nude mouse lung but not colon or subcutis. *Anticancer Res.* 15, 795-798, 1995.
11. ✓ Lef, D.N. MetaMouse models colon cancer metastasis with clinical potential. *BioWorld Today* 7, 1, January 8, 1996.
12. Murray, G., Duncan, M., O'Neil, P., Melvin, W., Fothergill, J. Matrix metalloproteinase-1 is associated with poor prognosis in colorectal cancer. *Nature Medicine* 2, 461-462, 1996.
13. An, Z., Wang, X., Willmott, N., Chander, S.K., Tickler, S., Docherty, A.J., Mountain, A., Millican, A.T., Morphy, R., Porter, J.R., Epemolu, R.O., Kubota, T., Moossa, A.R., and Hoffman, R.M. Conversion of highly malignant colon cancer from an aggressive to a controlled disease by oral administration of a metalloproteinase inhibitor. *Clinical & Experimental Metastasis* 15, 184-195, 1997.
14. Sun, F-X., Sasson, A.R., Jiang, P., An, Z., Gamagami, R., Li, L., Moossa, A.R., and Hoffman, R.M. An ultra-metastatic model of human colon cancer in nude mice. *Clinical & Experimental Metastasis* 17, 41-48, 1999.
15. Sasson, A.R., Gamagami, R., An, Z., Wang, X., Moossa, A.R., and Hoffman, R.M. Cimetine: An inhibitor or promoter of tumor growth? *International Journal of Cancer* 81, 835-838, 1999.
16. Rho, Y-S., Lee, K-T., Jung, J-C., Yoon, C., An, Z., Hoffman, R.M., and Chang, S-G. Efficacy of new platinum analog DPPE in an orthotopic nude mouse model of human colon cancer. *Anticancer Res.* 19, 157-162, 1999.
17. Rashidi, B., An, Z., Sun, F-X., Sasson, A., Gamagami, R., Moossa, A.R., Hoffman, R.M. Minimal liver resection strongly stimulates the growth of human colon cancer in the liver of nude mice. *Clinical & Experimental Metastasis* 17, 497-500, 1999.
18. Rashidi, B., Sun, F-X., Jiang, P., An, Z., Gamagami, R., Moossa, A.R., and Hoffman, R.M. A nude mouse model of massive liver and lymph node metastasis of human colon cancer. *Anticancer Res.* 20, 715-722, 2000.
19. Rashidi, B., An, Z., Sun, F-X., Moossa, A.R., and Hoffman, R.M. Antimetastatic intraoperative chemotherapy of human colon tumors in the livers of nude mice. *Clinical Cancer Res.* 6, 2464-2468, 2000.
20. Rashidi, B., Gamagami, R., Sasson, A., Sun, F-X., Geller, J., Moossa, A.R., and Hoffman, R.M. An orthotopic mouse model of rcmetastasis of human colon cancer liver metastasis. *Clinical Cancer Res.* 6, 2556-2561, 2000.

## Gene Therapy

1. Miki, K., Xu, M., An, Z., Wang, X., Yang, M., Al-Refaie, W., Sun, X., Baranov, E., Tan, Y., Chishima, T., Shimada, H., Moossa, A.R., Hoffman, R.M. Survival efficacy of the combination of the methioninase gene and methioninase in a lung cancer orthotopic model. *Cancer Gene Therapy* 7, 332-338, 2000.

2. Miki, K., Al-Refaie, W., Xu, M., Jiang, P., Tan, Y., Bouvet, M., Zhao, M., Gupta, A., Chishima, T., Shimada, H., Makuuchi, M., Moossa, A.R., and Hoffman, R.M. Methioninase gene therapy of human cancer cells is synergistic with recombinant methioninase treatment. *Cancer Res.* 60, 2696-2702, 2000.
3. Hasegawa, S., Yang, M., Chishima, T., Shimada, H., Moossa, A.R., Hoffman, R.M. *In vivo* tumor delivery of the green fluorescent protein gene to report future occurrence of metastasis. *Cancer Gene Therapy* 7, 1336-1340, 2000.
4. Djeha, A.H., Thomson, T.A., Leung, H., Scarle, P.F., Young, L.S., Kerr, D.J., Harris, P.A., Mountain, A., and Wrighton, C.J. Combined adenovirus-mediated nitroreductase gene delivery and CB1954 treatment: A well-tolerated therapy for established solid tumors. *Molecular Therapy* 3, 233-240, 2001.
5. Chen, C.T., Lin, J., Li, Q., Phipps, S.S., Jakubczak, J.L., Stewart, D.A., Skripchenko, Y., Forry-Schaudies, S., Wood, J., Schnell, C., and Hallenbeck, P.L. Antiangiogenic gene therapy for cancer via systemic administration of adenoviral vectors expressing secreted endostatin. *Human Gene Therapy* 11, 1983-1996, 2000.
6. Miki, K., Xu, M., Gupta, A., Ba, Y., Tan, Y., Al-Refaie, W., Bouvet, M., Makuuchi, M., Moossa, A.R., and Hoffman, R.M. Methioninase cancer gene therapy with selenomethionine as suicide prodrug substrate. *Cancer Research* 61, 6805-6810, 2001.
7. Kamiyama, M., Ichikawa, Y., Ishikawa, T., Chishima, T., Hasegawa, S., Hamaguchi, Y., Nagashima, Y., Miyagi, Y., Mitsuhashi, M., Hyndman, D., Hoffman, R.M., Ohki, S., and Shimada, H. VEGF receptor antisense therapy inhibits angiogenesis and peritoneal dissemination of human gastric cancer in nude mice. *Cancer Gene Therapy* 9, 197-201, 2002.
8. Pirocanac, E.C., Nassirpour, R., Yang, M., Wang, J-W., Nardin, S.R., Gu, J., Fang, B., Moossa, A.R., Hoffman, R.M., and Bouvet, M. Bax-induction gene therapy of pancreatic cancer. *J. Surg. Res.* 106, 346-351, 2002.

## GFP

1. Chishima, T., Miyagi, Y., Wang, X., Yamaoka, H., Shimada, H., Moossa, A.R., and Hoffman, R.M. Cancer invasion and micrometastasis visualized in live tissue by green fluorescent protein expression. *Cancer Res.* 57, 2042-2047, 1997.
2. Chishima, T., Miyagi, Y., Wang, X., Baranov, E., Tan, Y., Shimada, H., Moossa, A.R., and Hoffman, R.M. Metastatic patterns of lung cancer visualized live and in process by green fluorescent protein expression. *Clinical & Experimental Metastasis* 15, 547-552, 1997.
3. Chishima, T., Miyagi, Y., Wang, X., Tan, Y., Shimada, H., Moossa, A.R., and Hoffman, R.M. Visualization of the metastatic process by green fluorescent protein expression. *Anticancer Res.* 17, 2377-2384, 1997.
4. Chishima, T., Yang, M., Miyagi, Y., Li, L., Tan, Y., Baranov, E., Shimada, H., Moossa, A.R., Penman, S., and Hoffman, R.M. Governing step of metastasis visualized *in vitro*. *Proc. Natl. Acad. Sci. USA* 94, 11573-11576, 1997.
5. Chishima, T., Miyagi, Y., Li, L., Tan, Y., Baranov, E., Yang, M., Shimada, H., Moossa, A.R., and Hoffman, R.M. The use of histoculture and green fluorescent protein to visualize tumor cell host interaction. *In Vitro Cell. Dev. Biol.* 33, 745-747, 1997.
6. Yang, M., Hasegawa, S., Jiang, P., Wang, X., Tan, Y., Chishima, T., Shimada, H., Moossa, A.R., and Hoffman, R.M. Widespread skeletal metastatic potential of human lung cancer revealed by green fluorescent protein expression. *Cancer Res.* 58, 4217-4221, 1998.
7. Yang, M., Jiang, P., Sun, F.X., Hasegawa, S., Baranov, E., Chishima, T., Shimada, H., Moossa, A.R., and Hoffman, R.M. A fluorescent orthotopic bone metastasis model of human prostate cancer. *Cancer Res.* 59, 781-786, 1999.
8. Hoffman, R.M. Orthotopic transplant mouse models with green fluorescent protein-expressing cancer cells to visualize metastasis and angiogenesis. *Cancer and Metastasis Reviews* 17, 271-277, 1999.
9. Hoffman, R.M. Green fluorescent protein to visualize cancer progression and metastasis. *In: Methods in Enzymology, Green Fluorescent Protein*, Vol. 302, 20-31. P. Michael Conn, ed. Academic Press: San Diego, 1999.
10. Naumov, G.N., Wilson, S.M., MacDonald, I.C., Schmidt, E.E., Morris, V.L., Groom, A.C., Hoffman, R.M., and Chambers, A.F. Cellular expression of green fluorescent protein, coupled with high-resolution *in vivo* videomicroscopy, to monitor steps in tumor metastasis. *Journal of Cell Science* 112, 1835-1842, 1999.
11. Yang, M., Chishima, T., Baranov, E., Shimada, H., Moossa, A.R., and Hoffman, R.M. Green fluorescent protein: A new light to visualize metastasis and angiogenesis in cancer. *Proc. of SPIE, Biomedical Imaging: Reporters, Dyes, and Instrumentation*, Vol. 3600, 117-124, 1999.

12. Yang, M., Jiang, P., An, Z., Baranov, E., Li, L., Hasegawa, S., Al-Tuwaijri, M., Chishima, T., Shimada, H., Moossa, A.R., Hoffman, R.M. Genetically fluorescent melanoma bone and organ metastasis models. *Clinical Cancer Res.* 5, 3549-3559, 1999.
13. Yang, M., Chishima, T., Wang, X., Baranov, E., Shimada, H., Moossa, A.R., and Hoffman, R.M. Multi-organ metastatic capability of Chinese hamster ovary cells revealed by green fluorescent protein (GFP) expression. *Clinical & Experimental Metastasis*, 17, 417-422, 1999.
14. Hoffman, R.M. Visualization of metastasis in orthotopic mouse models with green fluorescent protein. *In: Relevance of Tumor Models in Anticancer Drugs Development*, Vol. 54, 81-87. Fiebig, H.H., Burger, A.M., eds. Dordrecht, The Netherlands: Kluwer Academic Publishers, 1999.
15. Yang, M., Baranov, E., Jiang, P., Sun, F-X., Li, X-M., Li, L., Hasegawa, S., Bouvet, M., Al-Tuwaijri, M., Chishima, T., Shimada, H., Moossa, A.R., Penman, S., Hoffman, R.M. Whole-body optical imaging of green fluorescent protein-expressing tumors and metastases. *Proc. Natl. Acad. Sci. USA* 97, 1206-1211, 2000.
16. Dove, A. Illuminating cancer models. *Nature Biotechnology* 18, 261, 2000.
17. Hoffman, R.M. Correspondence re: Initial stages of tumor cell-induced angiogenesis: Evaluation via skin window chambers in rodent models. *Journal of the National Cancer Institute* 92, 1445, 2000.
18. Yang, M., Baranov, E., Shimada, H., Moossa, A.R., and Hoffman, R.M. External optical imaging freely-moving mice with green fluorescent protein-expressing metastatic tumors. *Proc. of SPIE Conference*, 3921, 256-259, 2000.
19. Corrigendum, BioFeedback. *Biotechniques* 29, 544, September 2000.
20. Yang, M., Baranov, E., Moossa, A.R., Penman, S., Hoffman, R.M. Visualizing gene expression by whole-body fluorescence imaging. *Proc. Natl. Acad. Sci. USA* 97, 12278-12282, 2000.
21. Hasegawa, S., Yang, M., Chishima, T., Shimada, H., Moossa, A.R., Hoffman, R.M. *In vivo* tumor delivery of the green fluorescent protein gene to report future occurrence of metastasis. *Cancer Gene Therapy* 7, 1336-1340, 2000.
22. Rashidi, B., Yang, M., Jiang, P., Baranov, E., An, Z., Wang, X., Moossa, A.R., and Hoffman, R.M. A highly metastatic Lewis lung carcinoma orthotopic green fluorescent protein model. *Clinical & Experimental Metastasis* 18, 57-60, 2000.
23. Bouvet, M., Yang, M., Nardin, S., Wang, X., Jiang, P., Baranov, E., Moossa, A.R., Hoffman, R.M. Chronologically-specific metastatic targeting of human pancreatic tumors in orthotopic models. *Clinical & Experimental Metastasis* 18, 213-218, 2000.
24. Yang, M., Baranov, E., Li, X-M., Wang, J-W., Jiang, P., Li, L., Moossa, A.R., Penman, S., Hoffman, R.M. Whole-body and intravital optical imaging of angiogenesis in orthotopically implanted tumors. *Proc. Natl. Acad. Sci. USA* 98, 2616-2621, 2001.
25. Robinson, K. Imaging system captures whole-body GFP images. *Biophotonics International*, April, 54-55, 2001.
26. Pfeifer, A., Kessler, T., Yang, M., Baranov, E., Kootstra, N., Cheresch, D.A., Hoffman, R.M., and Verma, I.M. Transduction of liver cells by lentiviral vectors: Analysis in living animals. *Molecular Therapy* 3, 319-322, 2001.
27. Hoffman, Robert M. Visualization of GFP-expressing tumors and metastasis *in vivo*. *BioTechniques* 30, 1016-1016, 2001.
28. Hutchinson, E. Fluorescence imaging of tumour metastasis and angiogenesis. *The Lancet Oncology*, 2, 254, 2001.
29. McCann, J. Jellyfish protein gives new glow to tumor imaging. *Journal of the National Cancer Institute* 93, 976-977, 2001.
30. Lee, N.C., Bouvet, M., Nardin, S., Jiang, P., Baranov, E., Rashidi, B., Yang, M., Wang, X., Moossa, A.R., and Hoffman, R.M. Antimetastatic efficacy of adjuvant gemcitabine in a pancreatic cancer orthotopic model. *Clinical & Experimental Metastasis* 18, 379-384, 2001.
31. Hoffman, R.M. Green Fluorescent Protein for Metastasis Research. *In: Methods in Molecular Medicine*, Vol. 58: Metastasis Research Protocols, Vol. 2: Cell Behavior *In Vitro* and *In Vivo*, pp. 285-298. Brooks, S.A., and Schumacher, U., eds. Totowa, NJ: Humana Press Inc., 2001.
32. Hoffman, R.M. GFP-Expressing Metastatic-Cancer Mouse Models. *In: Tumor Models in Cancer Research*, pp. 99-112. Teicher, B., ed. Totowa, NJ: Humana Press Inc., 2002.
33. Kamiyama, M., Ichikawa, Y., Ishikawa, T., Chishima, T., Hasegawa, S., Hamaguchi, Y., Nagashima, Y., Miyagi, Y., Mitsunashi, M., Hyndman, D., Hoffman, R.M., Ohki, S., and Shimada, H. VEGF receptor antisense therapy inhibits angiogenesis and peritoneal dissemination of human gastric cancer in nude mice. *Cancer Gene Therapy* 9, 197-201, 2002.

34. Yang, M., Baranov, E., Wang, J-W., Jiang, P., Wang, X., Sun, F-X., Bouvet, M., Moossa, A.R., Penman, S., and Hoffman, R.M. Direct external imaging of nascent cancer, tumor progression, angiogenesis, and metastasis on internal organs in the fluorescent orthotopic model. *Proc. Natl. Acad. Sci. USA* 99, 3824-3829, 2002.
35. Bouvet, M., Wang, J-W., Nardin, S.R., Nassirpour, R., Yang, M., Baranov, E., Jiang, P., Moossa, A.R., and Hoffman, R.M. Real-time optical imaging of primary tumor growth and multiple metastatic events in a pancreatic cancer orthotopic model. *Cancer Research* 62, 1534-1540, 2002.
36. Hoffman, R.M. Green fluorescent protein imaging of tumor cells in mice. *Lab Animal* 31, 34-41, 2002.
37. Bouvet, M., Nardin, S.R., Burton, D.W., Lee, N.C., Yang, M., Wang, X., Baranov, E., Behling, C., Moossa, A.R., Hoffman, R.M., and Deftos L.J. Parathyroid hormone-related protein as a novel tumor marker in pancreatic adenocarcinoma. *Pancreas* 24, 284-290, 2002.
38. Schmitt, C.A., Yang, M., Fridman, J.S., Baranov, E., Hoffman, R.M., and Lowe, S.W. Senescence program controlled by p53 and p16<sup>INK4a</sup> contributes to the outcome of cancer therapy. *Cell* 109, 335-346, 2002.
39. Li, X-M., Wang, J-W., An, Z., Yang, M., Baranov, E., Jiang, P., Sun, F-X., Moossa, A.R., and Hoffman, R.M. Optically imageable metastatic model of human breast cancer. *Clinical & Experimental Metastasis*, 19, 347-350, 2002.
40. Hoffman, R.M. Watching real-time metastasis *in vivo*. *Trends in Molecular Medicine*, 8(7), 354-355, 2002.
41. Hoffman, R.M. Whole-body fluorescence imaging with Green Fluorescent Protein. *In: Methods in Molecular Biology*, Vol. 183: Green Fluorescent Protein: Applications and Protocols, pp. 135-148. Hicks, B.W., ed. Totowa, NJ: Humana Press, 2002.
42. Hoffman, R.M. *In vivo* imaging of metastatic cancer with fluorescent proteins. *Cell Death & Differentiation* 9, 786-789, 2002.
43. Zhou, J-H., Rosser, C.J., Tanaka, M., Yang, M., Baranov, E., Hoffman, R.M., Benedict, W.F. Visualizing superficial human bladder cancer cell growth *in vivo* by green fluorescent protein expression. *Cancer Gene Therapy* 9, 681-686, 2002.
44. Hoffman, R.M. Green fluorescent protein imaging of tumour growth, metastasis, and angiogenesis in mouse models. *Lancet Oncology* 3, 546-556, 2002.
45. Sun, F-X., Tohgo, A., Bouvet, M., Yagi, S., Nassirpour, R., Moossa, A.R., and Hoffman, R.M. Efficacy of camptothecin analog DX-8951f (Exatecan Mesylate) on human pancreatic cancer in an orthotopic metastatic model. *Cancer Research* 63, 80-85, 2003.
46. Wang, J-W., Yang, M., Wang, X., Sun, F-X., Li, X-M., Yagi, S., and Hoffman, R.M. Antimetastatic efficacy of oral 5-FU imaged by green fluorescent protein in real time. *Anticancer Research* 23, 1-6, 2003.
47. Yamamoto, N., Yang, M., Jiang, P., Tsuchiya, H., Tomita, K., Moossa, A.R., and Hoffman, R.M. Real-time GFP imaging of spontaneous HT-1080 fibrosarcoma lung metastases. *Clin. Exp. Metastasis* 20, 181-185, 2003.
48. John, C.M., Leffler, H., Kahl-Knutsson, B., Svensson, I., Jarvis, G.A. Truncated Galectin-3 Inhibits Tumor Growth and Metastasis in Orthotopic Nude Mouse Model of Human Breast Cancer. *Clinical Cancer Research* 9, 2374-2383, 2003.
49. Glinskii, A.B., Smith, B.A., Jiang, P., Li, X-M., Yang, M., Hoffman, R.M., Glinsky, G.V. Viable circulating metastatic cells produced in orthotopic but not ectopic prostate cancer models. *Cancer Res.* 63, 4239-4243, 2003.
50. Katz, M., Takimoto, S., Spivac, D., Moossa, A.R., Hoffman, R.M., Bouvet, M. A novel red fluorescent protein orthotopic pancreatic cancer model for the preclinical evaluation of chemotherapeutics. *J. Surg. Res.* 113, 151-160, 2003.
51. Katz, M.H., Bouvet, M., Takimoto, S., Spivac, D., Moossa, A.R., Hoffman, R.M. Selective antimetastatic activity of cytosine analog CS-682 in a red fluorescent protein orthotopic model of pancreatic cancer. *Cancer Res.* 63, 5521-5525, 2003.
52. Goodison, S., Kawai, K., Hihara, J., Jiang, P., Yang, M., Urquidi, V., Hoffman, R.M., and Tarin, D. Prolonged dormancy and site-specific growth potential of cancer cells spontaneously disseminated from non-metastatic breast tumors revealed by labeling with green fluorescent protein. *Clinical Cancer Res.* 9, 3808-3814, 2003.
53. Yamamoto, N., Yang, M., Jiang, P., Xu, M., Tsuchiya, H., Tomita, K., Moossa, A.R., and Hoffman, R.M. Real-time imaging of individual color-coded metastatic colonics *in vivo*. *Clin. Exp. Metastasis* 20(7), 633-638, 2003.

## Kidney

1. An, Z., Jiang, P., Wang, X., Moossa, A.R., and Hoffman, R.M. Development of a high metastatic orthotopic model of human renal cell carcinoma in nude mice: Benefits of fragment implantation compared to cell-suspension injection. *Clinical & Experimental Metastasis* 17, 265-270, 1999.

## Lung

1. Wang, X., Fu, X., and Hoffman, R.M. A new patient-like metastatic model of human lung cancer constructed orthotopically with intact tissue via thoracotomy in immunodeficient mice. *Int. J. Cancer* 51, 992-995, 1992.
2. Kuo, T-H., Kubota, T., Watanabe, M., Furukawa, T., Kase, S., Tanino, H., Nishibori, K., Saikawa, Y., Teramoto, T., Ishibiki, K., Kitajima, M., and Hoffman, R.M. Orthotopic reconstitution of human small-cell lung carcinoma after intravenous transplantation in SCID mice. *Anticancer Res.* 12, 1407-1410, 1992.
3. Wang, X., Fu, X., and Hoffman, R.M. A patient-like metastasizing model of human lung adenocarcinoma constructed via thoracotomy in nude mice. *Anticancer Res.* 12, 1399-1402, 1992.
4. Wang, X., Fu, X., Kubota, T., and Hoffman, R.M. A new patient-like metastatic model of human small-cell lung cancer constructed orthotopically with intact tissue via thoracotomy in nude mice. *Anticancer Res.* 12, 1403-1406, 1992.
5. Kuo, T-H., Kubota, T., Watanabe, M., Furukawa, T., Kase, S., Tanino, H., Saikawa, Y., Ishibiki, K., Kitajima, M., and Hoffman, R.M. Site-specific chemosensitivity of human small-cell lung carcinoma growing orthotopically compared to subcutaneously in SCID mice: the importance of orthotopic models to obtain relevant drug evaluation data. *Anticancer Res.* 13, 627-630, 1993.
6. Astoul, P., Wang, X., and Hoffman, R.M. "Patient-like" nude- and SCID-mouse models of human lung and pleural cancer (Review). *Int. J. Oncology* 3, 713-718, 1993.
7. Astoul, P., Colt, H.G., Wang, X., and Hoffman, R.M. A "patient-like" nude mouse model of parietal pleural human lung adenocarcinoma. *Anticancer Res.* 14, 85-92, 1994.
8. Astoul, P., Colt, H.G., Wang, X., Boutin, C. and Hoffman, R.M. A "patient-like" nude mouse metastatic model of advanced human pleural cancer. *Journal of Cellular Biochemistry* 56, 9-15, 1994.
9. Astoul, P., Wang, X., Colt, H.G., Boutin, C., and Hoffman, R.M. A patient-like human malignant pleural mesothelioma nude-mouse model. *Oncology Reports* 3, 483-487, 1996.
10. Colt, H.G., Astoul, P., Wang, X., Yi, E.S., Boutin, C., and Hoffman, R.M. Clinical course of human epithelial-type malignant pleural mesothelioma replicated in an orthotopic-transplant nude mouse model. *Anticancer Res.* 16, 633-640, 1996.
11. An, Z., Wang, X., Astoul, P., Danays, T., Moossa, A.R., and Hoffman, R.M. Interferon gamma is highly effective against orthotopically-implanted human pleural adenocarcinoma in nude mice. *Anticancer Res.* 16, 2545-2551, 1996.
12. Olbina, G., Cieslak, D., Ruzdijic, S., Esler, C., An, Z., Wang, X., Hoffman, R., Seifert, W., and Pietrzkowski, Z. Reversible inhibition of IL-8 receptor B mRNA expression and proliferation in non-small cell lung cancer by antisense oligonucleotides. *Anticancer Res.* 16, 3525-3530, 1996.
13. Yang, M., Hasegawa, S., Jiang, P., Wang, X., Tan, Y., Chishima, T., Shimada, H., Moossa, A.R., and Hoffman, R.M. Widespread skeletal metastatic potential of human lung cancer revealed by green fluorescent protein expression. *Cancer Res.* 58, 4217-4221, 1998.
14. Rashidi, B., Yang, M., Jiang, P., Baranov, E., An, Z., Wang, X., Moossa, A.R., and Hoffman, R.M. A highly metastatic Lewis lung carcinoma orthotopic green fluorescent protein model. *Clinical & Experimental Metastasis* 18, 57-60, 2000.
15. Hoffman, Robert M. Metastatic Orthotopic Mouse Models of Lung Cancer. In: *Methods in Molecular Medicine. Lung Cancer, Vol. 1: Molecular Pathology Methods and Reviews*, pp. 457-464. Driscoll, B., ed. Totowa, NJ: Humana Press, 2003.
16. Yamamoto, N., Yang, M., Jiang, P., Tsuchiya, H., Tomita, K., Moossa, A.R., and Hoffman, R.M. Real-time GFP imaging of spontaneous HT-1080 fibrosarcoma lung metastases. *Clin. Exp. Metastasis* 20, 181-185, 2003.

## Liver

1. Sun, F.X., Tang, Z.Y., Liu, K.D., Ye, S.L., Xue, Q., Gao, D.M., and Ma, Z.C. Establishment of a metastatic model of human hepatocellular carcinoma in nude mice via orthotopic implantation of histologically intact tissues. *Int. J. Cancer* 66, 239-243, 1996.
2. Sun, F-X., Tang, Z-Y., Liu, K-D., Xue, Q., Gao, D-M., Yu, Y-Q., Zhou, X-D., Ma, Z-C. Metastatic models of human liver cancer in nude mice orthotopically constructed by using histologically intact patient specimens. *J. Cancer Res. Clin. Oncol.* 122, 397-402, 1996.
3. Rashidi, B., An, Z., Sun, F-X, Li, X-M., Tang, Z.Y., Moossa, A.R., and Hoffman, R.M. Efficacy of intra-hepatectomy 5-FU on recurrence and metastasis of human hepatocellular carcinoma in nude mice. *Int. J. Cancer* 91, 231-235, 2001.
4. Wu, M., Mazurchuk, R., Chaudhary, N.D., Spermyak, J., Veith, J., Pera, P., Greco, W., Hoffman, R.M., Kobayashi, T., and Bernacki, R.J. High-Resolution Magnetic Resonance Imaging of the Efficacy of the Cytosine Analogue 1-[2-C-Cyano-2-deoxy- $\beta$ -D-arabino-pentofuranosyl]-N<sup>4</sup>-palmitoyl Cytosine (CS-682) in a Liver-Metastasis Athymic Nude Mouse Model. *Cancer Res.* 63, 2477-2482, 2003.

## Ovarian

1. Fu, X., and Hoffman, R.M. Human ovarian carcinoma metastatic models constructed in nude mice by orthotopic transplantation of histologically-intact patient specimens. *Anticancer Res.* 13, 283-286, 1993.
2. Astoul, P., Colt, H.G., Wang, X., and Hoffman, R.M. Metastatic human pleural ovarian cancer model constructed by orthotopic implantation of fresh histologically-intact patient carcinoma in nude mice. *Anticancer Res.* 13, 1999-2002, 1993.
3. Kiguchi, K., Kubota, T., Aoki, D., Udagawa, Y., Yamanouchi, S., Saga, M., Amemiya, A., Nozawa, S., Moossa, A.R., and Hoffman, R.M. A patient-like orthotopic implantation nude mouse model of highly metastatic human ovarian cancer. *Clinical & Experimental Metastasis* 16, 751-756, 1998.

## Pancreas

1. Fu, X., Guadagni, F., and Hoffman, R.M. A metastatic nude-mouse model of human pancreatic cancer constructed orthotopically from histologically intact patient specimens. *Proc. Natl. Acad. Sci. USA* 89, 5645-5649, 1992.
2. Furukawa, T., Kubota, T., Watanabe, M., Kitajima, M., and Hoffman, R.M. A novel "patient-like" treatment model of human pancreatic cancer constructed using orthotopic transplantation of histologically intact human tumor tissue in nude mice. *Cancer Res.* 53, 3070-3072, 1993.
3. An, Z., Wang, X., Kubota, T., Moossa, A.R., Hoffman, R.M. A clinical nude mouse metastatic model for highly malignant human pancreatic cancer. *Anticancer Res.* 16, 627-631, 1996.
4. Tomikawa, M., Kubota, T., Matsuzaki, S.W., Takahasi, S., Kitajima, M., Moossa, A.R., and Hoffman, R.M. Mitomycin C and cisplatin increase survival in a human pancreatic cancer metastatic model. *Anticancer Res.* 17, 3623-3626, 1997.
5. Bouvet, M., Yang, M., Nardin, S., Wang, X., Jiang, P., Baranov, E., Moossa, A.R., Hoffman, R.M. Chronologically-specific metastatic targeting of human pancreatic tumors in orthotopic models. *Clinical & Experimental Metastasis* 18, 213-218, 2000.
6. Lee, N.C., Bouvet, M., Nardin, S., Jiang, P., Baranov, E., Rashidi, B., Yang, M., Wang, X., Moossa, A.R., and Hoffman, R.M. Antimetastatic efficacy of adjuvant gemcitabine in a pancreatic cancer orthotopic model. *Clinical & Experimental Metastasis* 18, 379-384, 2001.
7. Bouvet, M., Wang, J-W., Nardin, S.R., Nassirpour, R., Yang, M., Baranov, E., Jiang, P., Moossa, A.R., and Hoffman, R.M. Real-time optical imaging of primary tumor growth and multiple metastatic events in a pancreatic cancer orthotopic model. *Cancer Research* 62, 1534-1540, 2002.
8. Bouvet, M., Nardin, S.R., Burton, D.W., Lee, N.C., Yang, M., Wang, X., Baranov, E., Behling, C., Moossa, A.R., Hoffman, R.M., and Deftos L.J. Parathyroid hormone-related protein as a novel tumor marker in pancreatic adenocarcinoma. *Pancreas* 24, 284-290, 2002.



9. Sun, F-X., Tohgo, A., Bouvet, M., Yagi, S., Nassirpour, R., Moossa, A.R., and Hoffman, R.M. Efficacy of camptothecin analog DX-8951f (Exatecan Mesylate) on human pancreatic cancer in an orthotopic metastatic model. *Cancer Research* 63, 80-85, 2003.
10. Katz, M., Takimoto, S., Spivac, D., Moossa, A.R., Hoffman, R.M., Bouvet, M. A novel red fluorescent protein orthotopic pancreatic cancer model for the preclinical evaluation of chemotherapeutics. *J. Surg. Res.* 113, 151-160, 2003.
11. Katz, M.H., Bouvet, M., Takimoto, S., Spivac, D., Moossa, A.R., Hoffman, R.M. Selective antimetastatic activity of cytosine analog CS-682 in a red fluorescent protein orthotopic model of pancreatic cancer. *Cancer Res.* 63, 5521-5525, 2003.

## Prostate

1. Fu, X., Herrera, H., and Hoffman, R.M. Orthotopic growth and metastasis of human prostate carcinoma in nude mice after transplantation of histologically intact tissue. *Int. J. Cancer* 52, 987-990, 1992.
2. An, Z., Wang, X., Geller, J., Moossa, A.R., and Hoffman, R.M. Surgical orthotopic implantation allows high lung and lymph node metastatic expression of human prostate carcinoma cell line PC-3 in nude mice. *The Prostate* 34, 169-174, 1998.
3. Yang, M., Jiang, P., Sun, F.X., Hasegawa, S., Baranov, E., Chishima, T., Shimada, H., Moossa, A.R., and Hoffman, R.M. A fluorescent orthotopic bone metastasis model of human prostate cancer. *Cancer Res.* 59, 781-786, 1999.
4. Wang, X., An, Z., Geller, J., and Hoffman, R.M. High malignancy orthotopic nude mouse model of human prostate cancer LNCaP. *The Prostate* 39, 182-186, 1999.
5. Glinskii, A.B., Smith, B.A., Jiang, P., Li, X-M., Yang, M., Hoffman, R.M., Glinsky, G.V. Viable circulating metastatic cells produced in orthotopic but not ectopic prostate cancer models. *Cancer Res.* 63, 4239-4243, 2003.

## Sarcoma

1. Yamamoto, N., Yang, M., Jiang, P., Xu, M., Tsuchiya, H., Tomita, K., Moossa, A.R., and Hoffman, R.M. Real-time imaging of individual color-coded metastatic colonies *in vivo*. *Clin. Exp. Metastasis* 20(7), 633-638, 2003.

## Stomach

1. Furukawa, T., Fu, X., Kubota, T., Watanabe, M., Kitajima, M., and Hoffman, R.M. Nude mouse metastatic models of human stomach cancer constructed using orthotopic implantation of histologically intact tissue. *Cancer Res.* 53, 1204-1208, 1993.
2. Furukawa, T., Kubota, T., Watanabe, M., Kitajima, M., and Hoffman, R.M. Orthotopic transplantation of histologically intact clinical specimens of stomach cancer to nude mice: Correlation of metastatic sites in mouse and individual patient donors. *Int. J. Cancer* 53, 608-612, 1993.
3. Furukawa, T., Kubota, T., Watanabe, M., Kuo, T-H., Kitajima, M., and Hoffman, R.M. Differential chemosensitivity of local and metastatic human gastric cancer after orthotopic transplantation of histologically intact tumor tissue in nude mice. *Int. J. Cancer* 54, 397-401, 1993.
4. Inada, T., Ichikawa, A., Kubota, T., Ogata, Y., Moossa, A.R., and Hoffman, R.M. 5-FU-induced apoptosis correlates with efficacy against human gastric and colon cancer xenografts in nude mice. *Anticancer Res.* 17, 1965-1971, 1997.
5. Hasegawa, S., Yang, M., Chishima, T., Shimada, H., Moossa, A.R., Hoffman, R.M. *In vivo* tumor delivery of the green fluorescent protein gene to report future occurrence of metastasis. *Cancer Gene Therapy* 7, 1336-1340, 2000.
6. Kamiyama, M., Ichikawa, Y., Ishikawa, T., Chishima, T., Hasegawa, S., Hamaguchi, Y., Nagashima, Y., Miyagi, Y., Mitsuhashi, M., Hyndman, D., Hoffman, R.M., Ohki, S., and Shimada, H. VEGF receptor antisense therapy inhibits angiogenesis and peritoneal dissemination of human gastric cancer in nude mice. *Cancer Gene Therapy* 9, 197-201, 2002.



## Drug Discovery

1. Furukawa, T., Kubota, T., Watanabe, M., Kuo, T.H., Kase, S., Saikawa, Y., Tanino, H., Teramoto, T., Ishibiki, K., Kitajima, M., and Hoffman, R.M. Immunotherapy prevents human colon cancer metastasis after orthotopic onplantation of histologically-intact tumor tissue in nude mice. *Anticancer Res.* 13, 287-291, 1993.
2. Furukawa, T., Kubota, T., Watanabe, M., Kuo, T.H., Kitajima, M., and Hoffman, R.M. Differential chemosensitivity of local and metastatic human gastric cancer after orthotopic transplantation of histologically intact tumor tissue in nude mice. *Int. J. Cancer* 54, 397-401, 1993.
3. Furukawa, T., Kubota, T., Watanabe, M., Kitajima, M., and Hoffman, R.M. A novel "patient-like" treatment model of human pancreatic cancer constructed using orthotopic transplantation of histologically intact human tumor tissue in nude mice. *Cancer Res.* 53, 3070-3072, 1993.
4. Kubota, T., Inoue, S., Furukawa, T., Ishibiki, K., Kitajima, M., Kawamura, E., and Hoffman, R.M. Similarity of Serum - Tumor Pharmacokinetics of Antitumor Agents in Man and Nude Mice. *Anticancer Res.* 13, 1481-1484, 1993.
5. Wang, X., Fu, X., Brown, P.D., Crimmin, M.J., and Hoffman, R.M. Matrix metalloproteinase inhibitor BB-94 (Batimastat) inhibits human colon tumor growth and spread in a patient-like orthotopic model in nude mice. *Cancer Res.* 54, 4726-4728, 1994.
6. Murray, G., Duncan, M., O'Neil, P., Melvin, W., Fothergill, J. Matrix metalloproteinase-1 is associated with poor prognosis in colorectal cancer. *Nature Medicine* 2, 461-2, 1996.
7. An, Z., Wang, X., Astoul, P., Danays, T., Moossa, A.R., and Hoffman, R.M. Interferon gamma is highly effective against orthotopically-implanted human pleural adenocarcinoma in nude mice. *Anticancer Res.* 16, 2545-2551, 1996.
8. Olbina, G., Cieslak, D., Ruzdijic, S., Esler, C., An, Z., Wang, X., Hoffman, R., Seifert, W., and Pietrzkowski, Z. Reversible inhibition of IL-8 receptor B mRNA expression and proliferation in non-small cell lung cancer by antisense oligonucleotides. *Anticancer Res.* 16, 3525-3530, 1996.
9. An, Z., Wang, X., Willmott, N., Chander, S.K., Tickle, S., Docherty, A.J., Mountain, A., Millican, A.T., Morphy, R., Porter, J.R., Epemolu, R.O., Kubota, T., Moossa, A.R., and Hoffman, R.M. Conversion of highly malignant colon cancer from an aggressive to a controlled disease by oral administration of a metalloproteinase inhibitor. *Clinical & Experimental Metastasis* 15, 184-195, 1997.
10. Inada, T., Ichikawa, A., Kubota, T., Ogata, Y., Moossa, A.R., and Hoffman, R.M. 5-FU-induced apoptosis correlates with efficacy against human gastric and colon cancer xenografts in nude mice. *Anticancer Res.* 17, 1965-1971, 1997.
11. Tomikawa, M., Kubota, T., Matsuzaki, S.W., Takahasi, S., Kitajima, M., Moossa, A.R., and Hoffman, R.M. Mitomycin C and cisplatin increase survival in a human pancreatic cancer metastatic model. *Anticancer Res.* 17, 3623-3626, 1997.
12. Chang, S-G., Kim, J.I., Jung, J-C., Rho, Y-S., Lee, K-T., An, Z., Wang, X., and Hoffman, R.M. Antimetastatic activity of the new platinum analog {Pt(cis-dach)(DPPE)2NO<sub>3</sub>} in a metastatic model of human bladder cancer. *Anticancer Res.* 17, 3239-3242, 1997.
13. Sasson, A.R., Gamagami, R., An, Z., Wang, X., Moossa, A.R., and Hoffman, R.M. Cimetine: an inhibitor or promoter of tumor growth? *International Journal of Cancer* 81, 835-838, 1999.
14. Rho, Y-S., Lee, K-T., Jung, J-C., Yoon, C., An, Z., Hoffman, R.M., and Chang, S-G. Efficacy of new platinum analog DPPE in an orthotopic nude mouse model of human colon cancer. *Anticancer Res.* 19, 157-162, 1999.
15. Woessner, R., An, Z., Li, X-M., Hoffman, R.M., Dix, R., Bitonti, A. Comparison of three approaches to doxorubicin therapy: free doxorubicin, liposomal doxorubicin, and  $\beta$ -glucuronidase-activated prodrug (HMR 1826). *Anticancer Res.* 20, 2289-2296, 2000.
16. Rashidi, B., An, Z., Sun, F-X., Moossa, A.R., and Hoffman, R.M. Antimetastatic intraoperative chemotherapy of human colon tumors in the livers of nude mice. *Clinical Cancer Res.* 6, 2464-2468, 2000.
17. Rashidi, B., An, Z., Sun, F-X., Li, X-M., Tang, Z.Y., Moossa, A.R., and Hoffman, R.M. Efficacy of intra-hepatectomy 5-FU on recurrence and metastasis of human hepatocellular carcinoma in nude mice. *Int. J. Cancer* 91, 231-235, 2001.
18. Lee, N.C., Bouvet, M., Nardin, S., Jiang, P., Baranov, E., Rashidi, B., Yang, M., Wang, X., Moossa, A.R., and Hoffman, R.M. Antimetastatic efficacy of adjuvant gemcitabine in a pancreatic cancer orthotopic model. *Clinical & Experimental Metastasis* 18, 379-384, 2001.

## Review

1. Hoffman, R.M. Orthotopic is orthodox: Why are orthotopic-transplant metastatic models different from all other models? *J. Cellular Biochem.* 56, 1-3, 1994.
2. Hoffman, R.M. Fertile Seed and Rich Soil: The development of patient-like models of human cancer by surgical orthotopic implantation of intact tissue. Update Series: Comprehensive Textbook of Oncology, 3: 1-10. Schimpff, S.C. et al., eds. Baltimore: Williams & Wilkins, 1996.
3. Hoffman, R.M. Fertile seed and rich soil: The development of clinically relevant models of human cancer by surgical orthotopic implantation of intact tissue. *In: Anticancer Drug Development Guide: Preclinical Screening, Clinical Trials, and Approval*, pp.127-144. Teicher, B., ed. Totowa, NJ: Humana Press, 1997.
4. Hoffman, R.M. Orthotopic metastatic mouse models for anticancer drug discovery and evaluation: a bridge to the clinic. *Investigational New Drugs* 17, 343-359, 1999.
5. Hoffman, R.M. Clinically Accurate Orthotopic Mouse Models of Cancer. *In: Methods in Molecular Medicine, Vol. 58: Metastasis Research Protocols, Vol. 2: Cell Behavior In Vitro and In Vivo*, pp. 251-275. Brooks, S.A., and Schumacher, U., eds. Totowa, NJ: Humana Press, 2001.
6. Hoffman, R.M. Green Fluorescent Protein for Metastasis Research. *In: Methods in Molecular Medicine, Vol. 58: Metastasis Research Protocols, Vol. 2: Cell Behavior In Vitro and In Vivo*, pp. 285-298. Brooks, S.A., and Schumacher, U., eds. Totowa, NJ: Humana Press Inc., 2001.
7. Hoffman, R.M. GFP-Expressing Metastatic-Cancer Mouse Models. *In: Tumor Models in Cancer Research*, pp. 99-112. Teicher, B., ed. Totowa, NJ: Humana Press Inc., 2002.
8. Hoffman, R.M. Green fluorescent protein imaging of tumor cells in mice. *Lab Animal* 31, 34-41, 2002.
9. Hoffman, R.M. Watching real-time metastasis *in vivo*. *Trends in Molecular Medicine* 8(7), 354-355, 2002.
10. Hoffman, R.M. Whole-body fluorescence imaging with Green Fluorescence Protein. *In: Methods in Molecular Biology, Vol. 183: Green Fluorescent Protein: Applications and Protocols*, pp. 135-148. Hicks, B.W., ed. Totowa, NJ: Humana Press, 2002.
11. Hoffman, R.M. Green fluorescent protein imaging of tumour growth, metastasis, and angiogenesis in mouse models. *Lancet Oncology* 3, 546-556, 2002.
12. Hoffman, R.M. Orthotopic mouse models of Cancer. *In: Anticancer Drug Development Guide Preclinical Screening, Clinical Trials, and Approval, 2<sup>nd</sup> Ed.*, pp. 183-212. Teicher, B., Andrews, P.A., eds. Totowa, NJ: Humana Press, 2004.



PATENT  
Docket No. 312762001530

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Ann MONOSOV and Xinyu Fu.

Serial No.: 09/023,232

Filing Date: 13 February 1998

For: NUDE MOUSE MODEL FOR HUMAN  
NEOPLASTIC DISEASE

Examiner: Ann Marie S. Wehbé

Group Art Unit: 1632

**SECOND DECLARATION OF ROBERT M. HOFFMAN**

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Dear Sir:

I, ROBERT M. HOFFMAN, declare as follows:

1. I am a Professor in the Department of Surgery at the University of California San Diego Medical Center and am Chairman of the Board and Chief Executive Officer of AntiCancer, Inc., the assignee herein. I obtained my Ph.D. in biology from Harvard University in 1971 and have been practicing in the field of cancer research since that time. I have held academic positions at Harvard Medical School and at the Weizmann Institute and have published about 300 articles on subjects related to cancer and metastasis. I am on the editorial boards of *AntiCancer Research*, of *Clinical Cancer Research* and of *In Vitro Cellular and Developmental Biology*. My *curriculum vitae* was submitted along with my previous declaration and may be referred to, as needed, by the Office.

2. I have supervised research at AntiCancer, Inc., which employs the surgical orthotopic implantation of intact tumor tissue as claimed in the herein application. The research has resulted in the publication of numerous papers establishing that the model successfully mimics, in an immunocompromised rodent, the clinical course of tumor growth and metastasis. The results using this model are dramatically superior to those obtained by Kyriazis, Otto, Wang or McLemore.

3. In my previous declaration, I submitted a summary of results obtained in this work done under my supervision which confirmed that the views expressed in the foregoing paragraph 2 are indeed correct. These results were provided as Exhibits 2 and 3 to my previous declaration and copies of these documents are enclosed with this declaration as Exhibit A for the convenience of the Examiner. I note that an objection was made to the citation of the standard reference work of Holland, James E., *et al.*, editor, Cancer Medicine, 5th ed., B.C. Decker, Inc., Hamilton, Ontario, Canada (2000), as describing the known clinical course of various tumors on the basis that this document post-dates the application date herein by a dozen years. However, the description in this document merely updates a description providing substantially the same results in earlier editions of this same reference work.

4. In order to confirm that the metastatic patterns observed with the AntiCancer MetaMouse shown in the previously submitted exhibits is consistent with the clinical pattern of metastasis known at the time, I have attached copies of the appropriate pages from an earlier edition of Cancer Medicine published in 1973. The cover page and front-piece showing the date are attached as Exhibit B.

- Page 1 of Exhibit 2 previously submitted showed that when bladder cancer related to the RT-4 cell line was employed, metastases were found in liver, pancreas, diaphragm,

omentum, iliac lymph nodes, superficial inguinal lymph nodes, and gastric lymph nodes; when the bladder cancer related to RT-10 was employed, metastases were found in the liver, lung, pancreas, spleen, diaphragm, and lymph nodes. Exhibit C, which is pages 1670 and 1674 of the 1973 Cancer Medicine edition, states on page 1674 that bladder tumors that invade the bladder wall metastasize most commonly to the pelvic nodes and to distant locations including lung, liver and bone. As noted, metastases were indeed found in the liver and lung as well as the regional lymph nodes in our model.

- As noted on page 2 of Exhibit 2, previously submitted, for colon cancer of various types, metastases were always found in the liver and often in the lungs as well as in the mesenteric lymph nodes. Exhibit D, which is pages 1597 and 1600-1601 of Cancer Medicine, states on page 1601 that liver and lungs are overwhelmingly the most frequent sites of distant metastases.
- As set forth on page 3 of Exhibit 2, in our model, pancreatic cancer showed consistent metastases in the liver and intestinal organs and often in the lungs (MIA PaCa, BxPC-3 and Pan-12-JCK). As noted on page 1561 of Exhibit E (pp. 1559-1561 of Cancer Medicine), regional lymph nodes and liver are the most common sites and intestinal organs and lungs are frequently involved.
- As shown on page 4 of Exhibit 2, our breast cancer models showed metastases to the axillary lymph nodes, lung, liver and bone for MDA-MB-435 and to the lung for AC-2468. Exhibit F (which includes pp. 1772-1774 of Cancer Medicine) states on page 1773 that the leading sites of distant metastases are the lymph nodes, lungs, red bone marrow, liver and bone.

- Exhibit 3 attached to my previous Declaration showed that renal cell carcinoma metastasized to lung, lymph nodes and liver; Exhibit G which includes page 1656 of Cancer Research states that the lungs, bones and liver are the most frequent sites involved in metastases of kidney cancer.

5. In my previous Declaration, I also included two papers which indicated the ability of the invention model to mimic lung cancer and provided the results of studies using this model. To summarize, the Rashidi paper showed extensive metastases in the immediate area including the contralateral lung, and the mediastinal lymph nodes. In 40% of the model animals heart metastases occurred and brain metastases occurred in 30%. According to the Yang paper, widespread metastasis was found throughout the skeleton, as well as in the collateral lung. Exhibit H contains copies of the relevant pages of the 1973 edition of Cancer Medicine relating to the clinical progression of this condition. As shown on page 1484, extension of the tumor into the chest wall is very common and, as shown on page 1485, metastases to the supraclavicular lymph nodes, brain, abdominal organs and bone are also common. Brain metastases are often found at autopsy as set forth on page 1489.

6. Attached as Exhibit I, for the convenience of the Office, is a summary of the metastatic locations of various tumors as set forth in Exhibits C through H. In my professional judgment, the results that we have obtained and that were submitted in my previous Declaration demonstrate an unexpected superiority of the claimed model in immunocompromised rodents. It is, of course, understood that there is considerable individual variation in metastatic patterns. Not all patients with colon cancer, for example, exhibit metastasis to the lungs, but such metastases are found in many. The rodent model shows similar individual variability, but in general mimics the patterns found in patients.

7. I am familiar with the paper cited as a primary reference, Kyriazis, *Cancer Research* (1981) 41:3995-4000, with which the comparisons in Exhibit A (Exhibit 2) were made. It is evident from the paper itself that no particular advantage is seen by the authors with regard to the value of using intact tissue. Because I have done extensive work in this field, I understand that the use of intact tissue in a subcutaneous model is considerably more convenient than the use of cell suspensions, since the use of intact tissue does not require pretreatment of the excised tumor. Based on this added convenience, I believe that Kyriazis, *et al.*, utilized intact tissue, not in order to improve the results obtained in the model, but rather in order to save time and avoid inconvenience.

8. I was directly involved in the development of the models that are claimed in the present application, since this development took place at AntiCancer. At the time, the inventors were involved in studying the growth of tumors *in vitro*. One of the approaches employed was to provide a matrix for tumor growth, typically as a three-dimensional collagen gel. It was observed by the inventors that when tumor cells were cultured in this way, *i.e.*, preserving a controlled three-dimensional structure, their response to test protocols and compounds and the general behavior of the tumor growth was much more consistent with what is observed *in vivo* than was the case when tumors were simply cultured as cell suspensions. It was based on these observations that the inventors realized that the use of intact tissue, with its pre-imposed three-dimensional structure, would be preferable to the use of cell suspensions in any kind of tumor model. It was also appreciated by the inventors that an orthotopic model might offer an advantage. Because of the observations made on three-dimensional matrices, the present inventors came to the conclusion that the inconvenience of the complex techniques required to utilize intact tissue in an orthotopic model was worth tolerating, since they believed that the



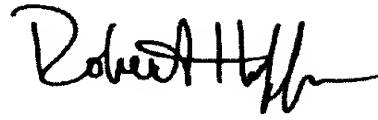
model might provide superior results as compared to other models known at the time. The results obtained when this inconvenience was tolerated and the present model was employed were indeed unexpectedly superior to what was contemplated. As shown in the results previously submitted, faithful reproduction of tumor progression in humans, including metastatic patterns was obtained.

9. It has been brought to my attention that the Office has taken the position that the work of Kyriazis in *Cancer Research*, cited above, observed metastases only in lymph nodes and lungs because other tissues were not examined. This is contradicted by the article itself which states that sections from various organs were fixed and examined histologically. As a worker in this field, I can testify that if histological examination had revealed metastases in other tissues, these would have been reported. Indeed, this is consistent with the observation made by the Office that in one case, metastasis was found in the diaphragm, and duly reported.

10. The value of the claimed tumor progression model is also evident from the high degree of commercial success that it has enjoyed. My company, AntiCancer, Inc., has received more than 100 service contracts from large pharmaceutical companies and other entities to employ the model as a contract service for testing of drugs and protocols. A significant portion of our company's income has been derived from such service contracts.

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Executed at San Diego, California, on 30 December 2003.

A handwritten signature in black ink, appearing to read "Robert Hoffman", with a stylized flourish at the end.

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ROBERT M. HOFFMAN